Ventricular haemodynamics in *Python molurus*: separation of pulmonary and systemic pressures

Tobias Wang[1],[*], Jordi Altimiras[2], Wilfried Klein[3] and Michael Axelsson[4]

[1]*Department of Zoophysiology, University of Aarhus, 8000 Aarhus C, Denmark,* [2]*Department of Biology, IFM, University of Linköping, Sweden,* [3]*Institute of Zoology, University of Bonn, Germany* and [4]*Department of Zoology, University of Gothenburg, Sweden*

*Author for correspondence (e-mail: tobias.wang@biology.au.dk)

Summary

Vascular pressure separation by virtue of a two-chambered ventricle evolved independently in mammals and birds from a reptilian ancestor with a single ventricle, and allowed for high systemic perfusion pressure while protecting the lungs from oedema. Within non-crocodilian reptiles, ventricular pressure separation has only been observed in varanid lizards and has been regarded as a unique adaptation to an active predatory life style and high metabolic rate. The systemic and pulmonary sides of the ventricle in *Python molurus* are well separated by the muscular ridge, and a previous study using *in situ* perfusion of the heart revealed a remarkable flow separation and showed that the systemic side can sustain higher output pressures than the pulmonary side. Here we extend these observations by showing that systemic blood pressure P_{sys} exceeded pulmonary pressure P_{pul} almost seven times (75.7 ± 4.2 *versus* 11.6 ± 1.1 cm H_2O). The large pressure difference between the systemic and pulmonary circulation persisted when P_{sys} was altered by infusion of sodium nitroprusside or phenylephrine. Intraventricular pressures, measured in anaesthetised snakes, showed an overlap in the pressure profile between the pulmonary side of the ventricle (cavum pulmonale) and the pulmonary artery, while the higher pressure in the systemic side of the ventricle (cavum arteriosum) overlapped with the pressure in the right aortic arch. This verifies that the pressure differences originate within the ventricle, indicating that the large muscular ridge separates the ventricle during cardiac contraction.

Key words: Reptile, snake, *Python molurus*, cardiovascular, blood pressure, ventricular pressure, vascular pressure separation.

Introduction

Vascular pressure separation by virtue of a two-chambered ventricle evolved independently in mammals and birds from a reptilian ancestor with a single ventricle. Complete separation of systemic and pulmonary blood flows allows for high systemic perfusion pressure and a more effective oxygen transport, while protecting the lungs from oedema (Burggren, 1982; Hicks, 1998; Burggren et al., 1998). Ventricular pressure separation and high systemic blood pressure have also evolved in varanid lizards and this trait is normally considered to be a unique adaptation to their active predatory life style and high metabolic rates (Burggren and Johansen, 1982; Burggren et al., 1998). Pressure separation within the heart of varanid lizards is accomplished by a well developed muscular ridge that separates the systemic side of the heart (cavum arteriosum, CA) from the pulmonary side of the heart (cavum pulmonale, CP) early in systole (Burggren and Johansen, 1982).

Ventricular pressure separation does not occur in the snakes *Vipera*, *Natrix* and *Thamnophis* (Johansen, 1959; Burggren, 1977), but the cardiac anatomy of pythons resembles that of varanid lizards and many descriptions of the heart from *Python*

molurus have emphasised the large muscular ridge and that the ventricular wall surrounding the systemic side of the heart is much thicker than the wall surrounding the pulmonary side (Jacquart, 1855; Webb et al., 1974; van Mierop and Kutsche, 1985; Farrell et al., 1998). Furthermore, the *in situ* perfused heart of *Python molurus* exhibits an extraordinary degree of flow separation and the systemic side of the ventricle can sustain higher output pressures than the pulmonary side (Wang et al., 2002). Given these observations, we decided to investigate blood pressures in the systemic and pulmonary circulations of conscious and chronically instrumented specimens of *Python molurus* and to measure the intraventricular pressures of anaesthetised animals.

Materials and methods

Experimental animals

The study was conducted on six captive bred specimens of Burmese pythons *Python molurus* L., body mass 1.0–2.2 kg (1.6 ± 0.2 kg, mean $\pm$ 1 S.E.M.) that had been maintained at The

University of Aarhus for more than a year. They were kept in individual chambers, equipped with a heating lamp on a L:D cycle of 12 h:12 h, allowing for behavioural thermoregulation and with free access to water. The snakes had been raised on a diet of mice and rats, but had been fasted for a minimum of 10 days before the experiments.

Surgeries and experimental protocols

The experiments consisted of two phases. First, animals were instrumented under general anaesthesia with catheters for measurements of systemic and pulmonary arterial blood pressures in fully recovered and conscious animals. Then, they were reanaesthetised for measurements of intraventricular pressures.

Instrumentation for measurements in recovered animals

For anaesthesia, a small plastic bag with saturated vapours of halothane was placed over the snake's head. When there was no longer any exhibited response to pinching their skin, it was possible to intubate the trachea with soft rubber tubing, so that the lungs could be artificially ventilated with 1.5% halothane mixed with air (Halothane vaporizer; Dräger, Lubeck, Germany) at a rate of 3 breaths min^{-1} and a tidal volume of 30 ml kg^{-1} using a ventilator (HI 665, Harvard Apparatus Inc., Holliston, MA, USA). A 5 cm ventrolateral incision was made immediately above and anterior to the heart, to allow a catheter (PE60 or PE90, filled with heparinised saline) to be placed occlusively in the cranial portion of the vertebral artery, from where it was advanced into the right aortic arch (RAo). The pulmonary circulation was cannulated by implanting an occlusive catheter (PE90) into the left pulmonary artery (LPA). In *Python*, the LPA is smaller than the right pulmonary artery, but this cannulation undeniably increased the resistance of the pulmonary circulation. Both catheters were exteriorised dorso-laterally and tied to the skin with several sutures. The incision was closed and the snakes were artificially ventilated with air until spontaneous lung breathing resumed. All snakes exhibited normal behaviour within the first 2 h after surgery had begun and were left undisturbed to recover in dark containers at room temperature (20–22°C). On the following day, the catheters were connected to pressure transducers and blood pressures of resting undisturbed snakes were measured over the next few hours.

To investigate whether the marked differences in pressure between the systemic and pulmonary circulation could be disrupted, we injected the vasodilator, sodium nitroprusside and the vasoconstrictor, phenylephrine *via* the arterial catheter (25 µg kg^{-1}; applied as a 1 ml bolus followed by an additional 1 ml of saline to flush the catheter).

Instrumentation for intraventricular measurements in anaesthetised animals

After completing the measurements in conscious snakes, individuals were anaesthetised by a slow infusion of 10–20 mg kg^{-1} sodium pentobarbital through the systemic catheter. When the animals no longer responded to having their skin pinched, the trachea was exposed for intubation and the lungs were artificially ventilated with 97% O_2, 3% CO_2, prepared by a Wösthoff gas mixing pump (Bochum, Germany) at a rate of 5 breaths min^{-1} and a tidal volume of approximately 50 ml kg^{-1} (Harvard Apparatus Respirator HI 665). The heart was then exposed by a ventral incision and the pericardium opened, so that i.v. catheters (Surflo, Terumo Medical Corporation, Elkton, MD, USA, connected to PE90) could be inserted into the CP and the CA. These catheters were placed directly through the ventricular wall and were secured in place by a thin suture (5–0) into the ventricular muscle tissue. When intraventricular pressures and pressures in the systemic and pulmonary arteries had been measured simultaneously, we performed some of the same drug injections as described above for the conscious animals. At the end of the experiment, the snakes were killed with an overdose of nembutal (200 mg kg^{-1} injected through the systemic catheter) and subsequent decapitation.

Measurements of blood pressures, data recording and statistics

Blood pressures were measured by connecting the catheters (PE60 or PE90, no longer than 60 cm) to Baxter Edward disposable pressure transducers (model PX600, Irvine, CA, USA). The signals from the pressure transducers were amplified by a preamplifier built in-house, and the amplified signal was collected at 100 Hz using a data acquisition system (MP100, Biopac, Goleta, CA, USA). All four pressure transducers were positioned level with the experimental animal's heart and calibrated several times a day against a common static water column.

All data are presented as means ± S.E.M. Statistical differences between systemic and pulmonary blood pressures and between values obtained on recovered and anaesthetised snakes were analysed on basis of a *t*-test and significant differences were accepted when $P<0.05$.

Results
Blood pressures and heart rate in conscious snakes

The blood pressure of the systemic circulation was substantially higher than that of the pulmonary circulation of recovered and conscious animals. This is shown by simultaneous measurement of pulmonary and systemic arterial pressure (P_{pul} and P_{sys}, respectively) from a resting and undisturbed snake in Fig. 1A; mean values for the five animals studied are shown in Fig. 1B. The systolic and diastolic pressures of the five animals are listed in Table 1. As shown in Fig. 1A, several animals exhibited transient changes in P_{sys} that appeared to correlate with pulmonary ventilation. The mean heart rate (f_H) of resting and undisturbed animals was 13.8±1.4 beats min^{-1}. In all individuals, P_{sys} exceeded P_{pul} by at least three times, and in some individuals there was a fivefold difference. The vascular pressure separation between the two circuits persisted when vascular resistance was modulated by injection of phenylephrine or sodium nitroprusside as shown in
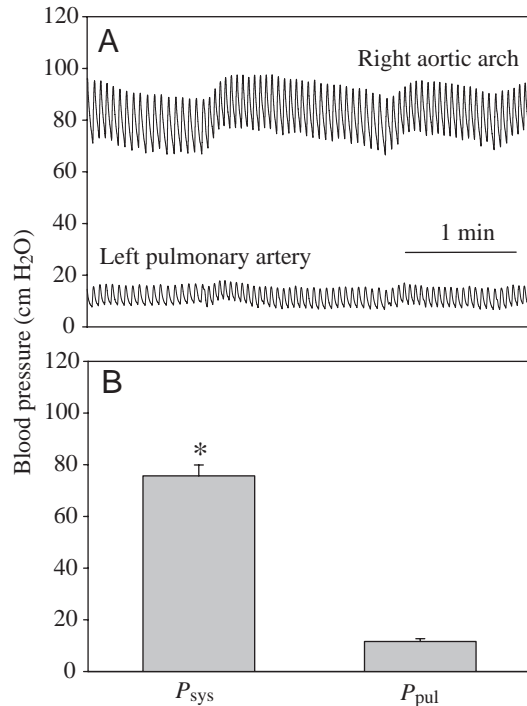
Fig. 1. (A) Simultaneous measurements of systemic (right aortic arch) and pulmonary (left pulmonary artery) arterial blood pressures (P_{sys} and P_{pul}, respectively) in a conscious and undisturbed specimen of *Python molurus*. (B) Mean P_{sys} and P_{pul} of five specimens. Values are means ± 1 S.E.M. (N=5). The asterisk indicates a significant difference between P_{sys} and P_{pul} (P<0.05; paired *t*-test). 1 cm H_2O=98 Pa.

Fig. 2. Effects of vascular injections of (A) phenylephrine (25 µg kg^{-1}) and (B) sodium nitroprusside (25 µg kg^{-1}) on systemic (right aortic arch) and pulmonary (left pulmonary artery) arterial blood pressures (P_{sys} and P_{pul}, respectively) in a conscious and undisturbed *Python molurus*. 1 cm H_2O=98 Pa.

Fig. 2. In all cases, these drugs elicited pronounced changes in P_{sys}, but there were no or small effects on P_{pul}. Similar relationships were recorded in four specimens (data not presented) and agitation of the snakes by manual disturbance was also ineffective as causing changes in P_{pul}.

Arterial and intraventricular pressures in anaesthetised snakes

The difference between P_{pul} and P_{sys} persisted during anaesthesia, but there were significant increases in both P_{pul}
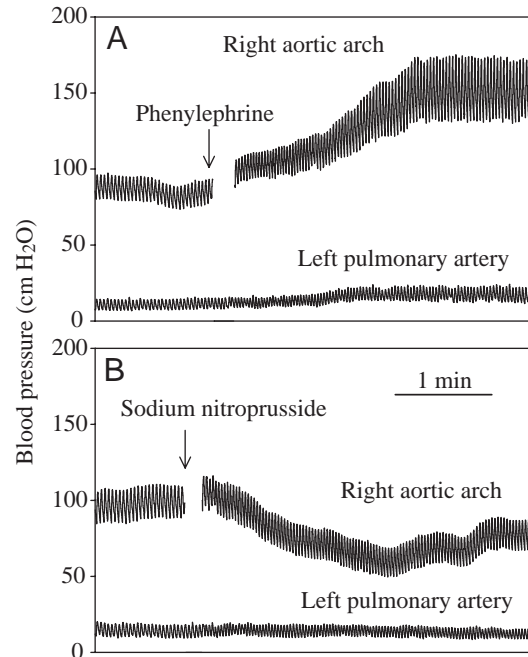
and P_{sys} compared to conscious animals (Table 1). Anaesthesia also elicited a large and significant increase in f_H from 13.8±1.4 to 33.7±2.2 beats min^{-1}.

In all six snakes studied, the measurements of intraventricular pressure showed an overlap in the pressure profile between the CP and the pulmonary artery, while the higher pressures in the CA overlapped with the pressures recorded in the right aortic arch (Fig. 3). In all individuals, it seemed that pressure in the CA increased before pressure rose in the CP. However, because of the much lower diastolic pressures in the pulmonary artery, ventricular ejection into the pulmonary circulation preceded ejection into the systemic circulation (Fig. 3).

Table 1. *Arterial blood pressures in the pulmonary circulation (left pulmonary artery) and the systemic circulation (right aortic arch) of conscious and anaesthetised* Python molurus

| | Pressure (cm H_2O) | | | | | |
| | Left pulmonary artery | | | Right aortic arch | | |
	Diastolic	Systolic	Mean	Diastolic	Systolic	Mean
Conscious	8.2±0.8	14.6±1.3	11.6±1.1	63.2±4.6*	87.6±4.1*	75.7±4.2*
Anaesthetised	19.7±4.7[†]	27.2±5.2[†]	23.0±4.9[†]	81.4±9.6*[,†]	102.0±11.5*[,†]	91.2±10.6*[,†]

1 cm H_2O=98 Pa.

Values are means ± S.E.M. (N=5 for the conscious animals, N=6 for anaesthetised animals).

*Significant difference between the pulmonary and systemic circulation; [†]significant difference between conscious and anaesthetised animals.
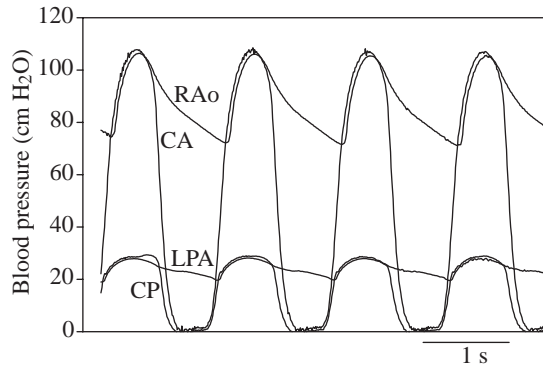
Fig. 3. Simultaneous measurements of arterial pressure in the systemic and pulmonary circulation and intraventricular pressure in an anaesthetised *Python*. RAo, Right aortic arch; CA, cavum arteriosum; LPA, left pulmonary artery; CP, cavum pulmonale. 1 cm H_2O=98 Pa.

Discussion

Systemic blood pressure of *Python molurus* is similar to mammals and considerably higher than in most other reptiles with the exception of varanid lizards (e.g. Johansen and Burggren, 1980; Burggren and Johansen, 1982; Shelton, 1993; Hicks, 1998; Burggren et al., 1998). Pulmonary arterial pressure is, on the other hand, lower than in any other reptiles, including varanid lizards. Recordings of intraventricular pressure established that the pressure differences are generated within the heart and the contraction of the CA seemed to precede CP, although ejection of blood into the pulmonary circulation occurred before the systemic valves opened (Fig. 3). The substantial pressure separation persisted in the face of large changes in systemic blood pressure induced by sodium nitroprusside or phenylephrine (Fig. 2). Collectively, these observations suggest that the CP and the CA are completely divided during the entire duration of systole so that the cardiac physiology of *Python molurus* strongly resembles that of varanid lizards (Millard and Johansen, 1974; Burggren and Johansen, 1982).

Although several aspects of the ventricular haemodynamics during systole remain speculative in *Varanus*, interpretation of pressure relationships is facilitated by reasonably good anatomical descriptions of their heart (e.g. Webb et al., 1971; Webb, 1979). The vertical septum is large and separates the CA from the left side of the heart. The CV is greatly reduced and may merely form the connection between the CA and PA. Webb (1979) even suggested that the vertical septum and the muscular ridge have merged to form a single structure, so that the CV actually has become part of the CP. While this may be an oversimplification based on nomenclature rather than haemodynamic function (Burggren and Johansen, 1982), it remains very likely that the functional separation of the CP and CA is accomplished by the free edge of the well-developed muscular ridge making direct contact to the ventricular wall early in systole. Detailed anatomical descriptions of the ventricle of *Python* are, unfortunately, not available, but the

structural similarities between varanid lizards and ophidians have been noted numerous times for more than a century (e.g. Jacquart, 1855; Robb, 1965; Webb et al., 1971, 1974; Webb, 1979; van Mierop and Kutsche, 1985; Farrell et al., 1998). Thus, as in *Varanus*, the ventricular wall surrounding the CA of *Python molurus* is much thicker than the wall surrounding the CP and the CA is extensively trabeculated compared to the CP (Farrell et al., 1998; T. Wang, personal observation). Given their anatomical and physiological similarities, the ventricular haemodynamics of *Python* and *Varanus* are probably very similar.

Ventricular pressure separation is consistent with the higher pressure and power generation of the systemic side of the *in situ* perfused heart from *Python molurus* (Wang et al., 2002). These differences are coupled with a considerable degree of flow separation between the systemic and pulmonary circulations (Wang et al., 2002). During *in situ* perfusion, right atrial inflow was predominantly directed to the pulmonary circulation, whereas left atrial inflow was directed primarily to the systemic circulation, suggesting that inflows from the two atria are well separated even during diastole. During diastole, flow separation probably results from the large right atrio–ventricular valve that directs right atrial inflow across the muscular ridge to the PA, while the left atrio–ventricular valve directs left atrial inflow to the base of the CA (White, 1968; Webb, 1979). Thus, it is most likely that the cardiac shunts that exist can be attributed to 'wash-out' of the small CV during the cardiac cycle (Heisler and Glass, 1985), suggesting that the magnitudes of the cardiac shunts in *Python*, as in *Varanus*, are relative small and that it does not change appreciably during different conditions (e.g. Berger and Heisler, 1977; Heisler et al., 1983; Ishimatsu et al., 1988). In *Python molurus*, systemic arterial oxygen levels are high even when metabolic rate is increased during digestion (Overgaard et al., 1999; Overgaard and Wang, 2002), which indicates that the degree of right–left shunt is rather small.

Our study shows that pronounced ventricular pressure separation is not restricted to the specialised group of varanid lizards, and although unlikely to be common among squamates, it may exist in more species than previous acknowledged. Ventricular pressure separation, however, does not occur in the snakes *Vipera berus, Natrix* and *Thamnophis* (Johansen, 1959; Burggren, 1977) and large cardiac shunts have been reported for sea snakes and rattlesnakes (Lillywhite and Donald, 1989; Wang et al., 1998). The development of high systemic blood pressure has often been linked to the higher cardiac output and increased capillary density that are associated with evolution of increased metabolic rate. To protect the lungs from oedema and possible structural damage (e.g. Burggren, 1982), ventricular division and pressure separation become necessary, while the further reduction of cardiac shunts associated with division also improves systemic oxygen delivery and augments maximal oxygen consumption (Wang and Hicks, 2002). As with the independent evolution of divided ventricles in endothermic birds and mammals, ventricular separation in *Varanus* is often correlated with their

active life style, high exercise capabilities and high metabolic rate (e.g. Burggren et al., 1998). *Python* is a typical inactive sit-and-wait predator that can ingest large prey (e.g. Secor and Diamond, 1995), and we have proposed that functional separation of the ventricle is related to the high oxygen consumption during digestion (Wang et al., 2002). Alternatively, it is possible that ventricular pressure separation in *Python molurus* is related to its use of shivering thermogenesis during egg incubation, which results in large and prolonged metabolic increments (Hutchison et al., 1966; Vinegar et al., 1970).

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